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Characterization of Withania somnifera dunal Powder Materials and analysis of Heavy Metal

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Abstract

Formulation of heavy metals is critical process in arsenic, mercury, lead, zinc and iron due to toxic or polluted environmental conditions, manufacturing processes during the final stage of transport and storage conditions. Ashwagandha is an important drug of medicine. It is found throughout the dry part of Ashwagandha. It play an important role for achievement good health in short duration and used to treatment various disorders like Vata roga, Unmad Vajikara temporary disease. According to WHO heavy metal concentration of herbal medicine definitely control permissible limit for cadmium was 0.3 ppm. Lead 10 ppm, arsenic 10 ppm, mercury 1 ppm. API has taken the initiative & implemented a few measure to control heavy metal concentration in herbal product. Acceptable limit is Lead 10 ppm, Arsenic 3 ppm, Mercury 1 ppm, Cadmium 0.3 ppm by as per WHO.

Introduction

Ashwagandha (*Withania somnifera L*) is an herbal plant belonging to the family Solanaceae. It also called as Indian ginseng or winter cherry (Paul et al., 2021). Ashwagandha's flowers having fruits with numerous seeds that easily identified from tropical and subtropical areas of South Africa, India and also China (Dar et al., 2015). It roots extract used to formulation in numerous materials like Churn and other extract. Herbal preparations are essential in assess the quality of drugs, based on the

concentration of their active principles (Yadav et al 2008). Evaluation of herbal property is a fundamental requirement of industry and other organization dealing with Ayurveda herbal products. The use of botanicals drug and other products derived from plants by the public is forcing on the health to develop standard quality and manufacture in the market. *Withania somnifera* plant part is used as antioxidant, sedative, pain reliever, memory enhancer, anti-neoplastic, anti-microbial, and anti-inflammatory agent in India (Narinderpal et al., 2013).

First time JAMA (*Journal of the American Medical Association* put some evidence that ayurvedic herbal products have heavy metal higher than their limit and consumer taking ayurvedic remedies (Saper et al 2004). In last two decades a large number of articles reported excessive toxic effect from heavy metals. Heavy metals include Lead, Mercury, Cadmium, Arsenic, or Thallium that reported in various Herbal medicinal plant products. (Ozdilek *et al*., 2007, Sahu *et al.,* 2007). *Withania somnifera* root metals are exceeds in plants and animals and causes a variety of acute and chronic disease in different way. The dietary supplements of essential elements provide a protection of human from heavy metals danger effect to As, Hg, Cd, Pb), while their deficiency may increase chronic disease (Okem et al., 2014). The heavy metals are causes more diseases from intake large amount in food. On the basis of above statement present study was conducted for the screening of heavy metals from the selected different sample of *W. somnifera* root part.

Materials and method

Macroscopic observation of roots of *Withania somnifera* Daunl was done. It comprised of shape, size, surface characteristics, texture, color, consistency, odor, taste, etc. Transverse sections of *Withania somnifera* Daunl *roots* were taken by using a section cutting method. Permanent mount of stem was prepared using saffranin fast green stain by double staining technique. 10g of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish. It was dried at 105˚C for 5 hours and at 1 hour interval until difference two successive weighing corresponded to not more than 0.25%. Two fresh samples of

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Withania somnifera herbal powder was collected from vindhya area's Mirzapur market. The fresh collected sample was stored under 4°C temperature. The collected samples was identified by Dr. Anil Kumar Singh, Professor Department of Dravya Guna, IMS BHU,Varanasi.

Table 1: Collected different Aswagandha samples from Vindhya region of Uttar Pradesh

Sample	Batch No	Mfg Date	Mfg Lic No,
Sample-T	335	9/11	A-2591/96
sample D	ALO129	09/12	A-5467/32

Crude fiber content

Procedure for water soluble extractive was followed for the determination of alcohol soluble extractive but 90% ethanol was used instead of chloroform water. Crude fiber contents in the bark samples were estimated according to the method described by Maynard. 2g of oven dried bark powder was transferred to 500ml conical flask and 200ml 0.255N H_2SO_4 was added to it. The contents were boiled for 30 minutes with bumping chips on hot plate. The flask was cooled and the contents filtered through muslin cloth. The residue was washed several times with hot distilled water. The residue thus obtained boiled with 200ml, 0.313N NaOH (1.25g of NaOH dissolved in 100ml distilled water). The contents were filtered through muslin cloth and the residue washed with 25ml, 1.25% H_2SO_4, three portions of water 50ml each and 25ml alcohol. The residue was removed and transferred to pre-weighed Ashing dish (W_1). The residue was dried at 130 ± 2^0C for 2hr. Ashing dish was cooled and weighed (W_2). The residue was ignited at 600 ± 15^0C. Ashing dish was cooled and weighed (W_3). Crude fiber contents in the bark samples were calculated by using following formula and expressed g.$100g^{-1}$ of dry weight. To few ml of filtrates, 1 to 2ml of dragendroff's reagent was added. A prominent yellow precipitate indicates the test is positive (Singh B, 2001).

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Crude fiber content = [Loss in weight on ignition (W2 −W1) − (W3 −W1)/ Weight of the sample] X 100

Thin Layer Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent. Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample of analysis on the same plate (Tewari et al., 2022). A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Detection and estimation of heavy metals

Samples were digested by the wet digestion method. 10ml of nitric acid was added to 2g of accurately weighed dried sample in a 100ml beaker and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5ml of concentrated nitric acid was added and heated for additional 30 min at 95°C. The last step was repeated and the solution was reduced to about 5ml without boiling. The sample was cooled again and 2ml of deionizer water and 3ml of 30% hydrogen peroxide was added. With the beaker covered, the sample was heated gently to start the peroxide reaction. If effervescence becomes excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1 ml increments, followed by gentle heating until the effervescence was subsides. 5ml of concentrated hydrochloric acid and 10ml of deionized water was added and the sample was heated for additional 15 min without boiling. The sample was cooled and filtered through a Whatman No. 42 filter paper and diluted to 50ml

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with deionized water (Kumar Sukender et al, 2012).

Digested samples were analyzed for Pb, Cd, and Zn using flame atomic absorption spectrophotometer. The 1000 ppm standard solutions of elements were diluted in five different concentrations to obtain calibration curve for quantative analysis. All the measurements were run in triplicate for the samples and standard solutions.

Angle of repose

The internal angle between the surface of the pile of powder and the horizontal surface is known as the angle of repose. The powder is passed through funnel fixed to a below the funnel on the table. The height and the radius of the pile were measured. Angle of repose of the powder was calculated using the formula

Angle of repose= tan-1(h/r)

Where,

h=height of the pile

r = radius of the pile

Table 2: Different properties are used in the *W. somnifera* analysis under laboratories condition

S.No.	Flow Properties	Angle of Repose	Compressibility Index (%)	Hausner Ratio
1.	Excellent	25-30	<10	1.00-1.11
2.	Good	31-35	11-15	1.12-1.18
3.	Fair	36-40	16-20	1.19-1.25
4.	Possible	41-45	21-25	1.26-1.34
5.	Poor	45-46	26-31	1.35-1.45
6.	Very Poor	55-56	32-37	1.46-1.59
7.	Very Very poor	>66	>38	>1.6

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Result and Discussion

W. somnifera have tap roots and main roots bear fiber like secondary roots. Their outer surface was buff to gray yellow with longitudinal wrinkles. The stem bases were variously thickened, cylindrical, green and have longitudinal wrinkles. *W. somnifera* root was showing following tissue systems: Cork exfoliated or crushed, when present isodiamatric and non-lignified, cork cambium of 2-4 diffused rows of cells. Secondary cortex have about twenty layers of compact parenchymatous cells, Phloem consists of sieve tubes, companion cells, phloem parenchyma, Cambium 4-5 rows of tangentially elongated cells. Secondary xylem hard forming a closed vascular ring separated by multi seriate medullary rays, a few xylem parenchyma arranged with bordered pits and horizontal perforations. There was starch grains abundant, simple, mostly spherical reniform, oval with central hilum. The W. *somnifera* drugs was prepared from dried roots of plant, it converted into small pieces, 10.0-17.5 cm long and 6-12 mm in diameter. The powdered pieces of roots were showing dark brown with creamy interior with straight, un-branched and conical structure (Balkrishna et al., 2020).

Table 3: Recorded different physical-chemical parameter of *W. somnifera*

S.N.	Parameters	Selected Sampls	Sample T	Sample D
1.	% Loss on drying	10.56 %	9.43 %	7.53 %
2.	Total Ash value	6.5 %	5.9 %.	5.3%
3.	Acid Insoluble Ash value	2.3%	1.9 %	1.6 %
4.	Water Soluble extract value (%w/w)	2.4 %	2.1 %	1.9%
5.	Alcohol Soluble Extract value (%w/w)	1.5 %	1.2 %	0.9 %
6.	Crude fiber Contents	5.0 %	4.2 %	4.0 %
7.	pH	7.10	7.80	8.40

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Table 4: Recorded different parameters of *W. somnifera* Churna development

S.No.	Parameters	Selected Sampls	Sample T	Sample D
1.	Bulk density (g/ml)	0.38	0.41	0.47
2.	Tap density (g/ml)	0.46	0.50	0.55
3.	Angle of repose (h/r)	43	39	35
4.	Compressibility	24	20	14
5.	Hausner	1.45	1.25	1.16

Table 5: Recorded different parameters in determination of organoleptic properties

S.No.	Parameters	Vindhyachal Area Sample	Sample T	Sample D
1.	**Colour**	Pinkish brown	Pale brown	Yellowish white
2.	**Odor**	Characteristics	Characteristics	Characteristics
3.	**Taste**	Slightly bitter	Slightly bitter	Slightly bitter

Detection & Estimation of Heavy Metal

Nitric acid, hydrochloric acid, sulphuric acid, hydrogen peroxide, sodium borohydride and stannous chloride were of analytical grade (E. Merck). The water used in all experiment was ultrapure water obtained from Milli-Q-water purification system (Ranken Rion Ltd, India). The standard solutions were prepared in five different concentrations to obtain calibration curve by diluting stock solutions (CPA Ltd) of 1000ppm of each element immediately before use. Atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) equipped with a deuterium lamp for background correction was used for determination of trace elements and heavy metals. The hollow-cathode lamps for Zn, Cd, (ECIL) and Pb (Photon) were employed as radiation source. The flames used were

air/acetylene and N_2O/acetylene. Nitrogen was used as carrier gas.

S.No.	Name of heavy metals	Vindhyachal Area Sample	Sample T	Sample D
1.	Zn	1.520ppm	1.987ppm	1.243ppm
2.	Cd	0.397ppm	0.350ppm	0.305ppm
3.	Pb	11.657ppm	10.27ppm	9.12ppm

The churna was extracted in ethanol and water hot percolation process. The extracts were collected and evaporated to dryness and cooled in a desiccators and the residue was subjected to photo chemical analysis.

The alcoholic extract was treated with tartaric acid and neutralized with ammonia and extract with chloroform. The chloroform extract was evaporated to dryness. The residue was dissolved in Methyl Alcohol and was spotted over silica gel film, the chromate plates were developed by using the chloroform & methanol (6:4) solvent system (Beckett et al 2016). Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10mm deep, close the tank and allow standing for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5mm wide, from the vertical sides of the plate Apply the solutions being examined in the form of circular spots about 2 to 6mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20mm to the sides; the spots should be 15mm apart. Close the tank and allow standing at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualize as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

Conclusions

In the present study selectesd samples of Ashwagandha powder (vindhya area sample, and two marketed samples) are investigated for physico-chemical, organoleptic character and powder characterization. In the result physico chemical parameter of this entire sample are observed similar to the API. In the present work Ashwagandha plant was analyzed for the toxic elements lead, cadmium, and zinc. Procedures were standardization and suitable method was selected for analysis. The Lead, cadmium content in the plant powder was slightly above the permitted level. The zinc content of ashwagandha plant powder is a normal limit. So quality of the collected vindhya area sample is better than the market samples. This Ashwagandha plant powder is used to both male and female participants safely for eight weeks. The usage of ashwagandha root extract in healthy male and female participants was shown to be safe, tolerable, and protect to any unintended harmful effects.

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